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# FORMULATION AND EVALUATION OF HERBAL GEL OF PLANT LEPTADENIA RETICULATE FOR ANTIFUNGAL ACTIVITY

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## ABSTRACT

The study investigates the therapeutic potential of Leptadenia reticulata, an important plant in Ayurvedic medicine known for its antimicrobial and antioxidant properties. The research aimed to explore the efficacy of ethanolic extracts of L. reticulata leaves in comparison to standard antimicrobial agents such as ciprofloxacin and amphotericin, and standard antioxidant compounds like Butylated Hydroxyl Anisole and L-ascorbic acid. The findings revealed that the extracts exhibit significant antimicrobial and antioxidant activities, suggesting their potential use in developing herbal medicinal products. The study emphasizes the importance of public education on the benefits of medicinal plants to promote their acceptance and integration into modern healthcare practices. Additionally, the research highlights the role of L. reticulata in the Ayurvedic industry for formulating and marketing drugs with international acceptance. The physicochemical evaluation of herbal gel formulations, including tests for homogeneity, spreadability, pH, and viscosity, demonstrated favorable results, supporting the viability of these formulations for therapeutic use. This research contributes to the broader field of ethnomedicine and pharmacognosy, advocating for the continued exploration and utilization of medicinal plants in traditional and contemporary medicine

**Keywords:** Anti-Fungal Activity, Herbal Gel, Leptadenia Reticulata , Evaluation Test.

## I. INTRODUCTION

Ayurveda, the ancient system of medicine that originated in India, uses various parts of the plant L. reticulata (also known as Limnophila reticulata) for treating a range of health conditions. This plant is valued for its therapeutic properties and is used in different forms, such as decoctions, pastes, and powders. The specific ailments it can address include digestive disorders, respiratory issues, inflammation, and more. Each part of the plant—leaves, roots, fruits, and stem—has unique properties that contribute to its overall medicinal efficacy [1]. Most studies on medicinal plants are pertaining to organic constituent's viz., essential oils, glycosides, alkaloids, phenols, flavonoids, vitamins, antioxidants compounds and their pharmacological activity together with toxic effects [2]. Medicinal plants indeed present significant opportunities for alternative remedies, particularly in rural areas where access to modern medical facilities may be limited. These plants have been traditionally used to prevent and treat various human diseases, leveraging their natural therapeutic properties (svarnajivantz) [3]. Herbs are more compatible with body because of their effects; therefore they are more suitable, especially in case of long consumption [4]. The utilization of plants for their therapeutic value has been known to mankind from times immemorial and has played an essential role in the various traditional systems of medicine including [5]. The history of medicinal plants is indeed parallel to the history of human beings. Since ancient times, people have relied on native plants to fulfill their basic needs, including nutrition, shelter, and medicine. Here are some key points highlighting the significance of medicinal plants [6]. Plants are source of inspiration to halt the emerging infections and diseases [7]. Medicinal plants have indeed been used indigenously for various purposes throughout the globe, including performing crucial roles in traditional medicinal systems. Despite their significance, only a small fraction of these plants have been scientifically analyzed for their potential, leaving a vast field of unexplored opportunities [8]. Leptadenai reticulata: Leptadenia reticulata (Retz.) Wight. & Arn. (Family Asclepiadaceae) is a climber, it is commonly known as Jivanti, Swarnjivanti or Dodi. Leptadenia reticulata is found to be an important plant in ethanobotanical studies. According to ayurveda it is tonic and gives general strength to body and it is used traditionally for several indications. It is considered as an important drug in Ayurveda since 4500 BC. In Atharva Veda this plant is described as life and strength giver [9].

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The various reports on its multiple uses in curing several diseases such as hematopoiesis, emaciation, cough, dyspnoea, fever, burning sensation, and night blindness draw the attention for utilization of this plant as drugs. It is regarded as good cure for tuberculosis and effectively used for several ear and nose problems. 50 % methanolic extract of this plant is having antibacterial activity and is used for the treatment of skin infection and wounds [10]. Leptadenia reticulata is also known as Jivanti (or Jiwanti) because of its nourishing property for every part of the body. Jivanti specially corrects the metabolism, digestive system and enhance the health status of the body. It is distributed in subtropical and tropical parts of Asia and Africa, Burma, Sri Lanka, Malayan peninsula, Philippines, Mauritius and Madagascar [11]. Medicinal plants are a rich and largely untapped resource for discovering new drugs. They offer a diverse range of bioactive compounds that can serve as leads for the development of novel pharmaceuticals. Here's an in-depth look at how these plants are utilized and analyzed for their therapeutic potential [12]. Taxonomical Classification:

**Table No.1** Taxonomical classification

| |
|---|
| Kingdom :Plantae |
| Phylum :Tracheophyta |
| Class: Magnoliopsida |
| Order :Gentianales |
| Family: Apocynaceae |
| Genus: Leptadenia |
| Species: Leptadenia pyrotechnica |

## II. METHODOLOGY

### 2.1.EXTRACTION

Preparation of Ethanolic Extract of Rubia cordifolia:

● The roots of Rubia cordifolia were carefully selected washed to remove impurities and shade dried. ● The dried material was reduced to fine powder in the mechanical grinder. ● The fine powder was passed through sieve no.43 and stored in an airtight container for further use. ● About 100 gm of powdered material was extracted with ethanol as a solvent by hot extraction method using Soxhlet apparatus. ● The extraction was continued until the solvent in the thimble became clear then few drops of solvent were collected in the test tube during the completion of the cycle and chemical test of the solvent was performed. ● After each extraction, the extract was evaporated to dryness in rotary vacuum evaporator. ● Moreover, some part of the extract was preserved for preliminary Phytochemical screening for the detection of various plant constituents and rest extract was used for formulation of batch.

### 2.2.PRELIMINARY PHYTOCHEMICAL INVESTIGATION

The ethanolic extract was subjected to qualitative chemical investigation. The following procedures were adopted to test for the presence of various phytochemical constituents in the extract. Most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins, saponins and glycosides. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs. The following procedures were adopted to test for the presence of various chemical constituents in extract.

1. TEST FOR SAPONINS Foam test A small amount of extract taken in a test tube with little quantity of water. Shake vigorously. Appearance of foam persisting for 10 minutes indicates presence of Saponin.

2. TEST FOR ALKALOIDS a) Mayer's test: 2-3 ml of filtrate with few drops of Mayer's reagent gives ppt. b) Wagner's test: 2-3 ml of filtrate with few drops of Wagner's reagent gives Reddish brown colour.

3. TEST FOR TANNINS Ferric chloride test: To the alcoholic solution of the extract add few drops of neutral ferric chloride solution. Appearance of green colour indicates presence of Tannins.

4. TEST FOR STEROIDS Liebermann's reaction: Mix 3 ml extract with 3 ml acetic anhydride. Heat and cool. Add few drops of conc. $H_2SO_4$. Blue color appears.

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5. TEST FOR FLAVANOIDS Alkaline reagent test: Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute acid. 6. TEST FOR TERPENOIDS Salkowski reaction: To 2 ml of extract, add 2 ml chloroform and 2 ml of conc. H2SO4. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

7. TEST FOR REDUCING SUGAR Benedict's test: Mix equal volume of Benedict's reagent and test extract in test tube. Heat in boiling water bath for 5 min. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution. 8. TEST FOR PROTEINS Biuret test a) Add 2ml of Biuret reagent to 2ml of extract. Shake well and warm it on water bath. Appearance of red or violet colour indicates presence of proteins. b) To 3 ml. extract add 4% NaOH and few drops of 1% CuSO4 solution. Violate or pink colour appears..

**Preformulation study**

Preformulation studies are needed to ensure the development of a stable as well as effective and safe dosage form. It is a stage of development during which the pharmacist characterizes the physic-chemical properties of the drug substances and its interaction with various formulation components. Goals of Preformulation study: • To determine the necessary physicochemical parameter of a new drug substance. • To establish its incompatibility with excipients of formulation.

**Experimental Design:**

**Formulation of Herbal Gel:**

**Preparation of herbal gel:**

**1. Selection of excipients:**

Rubia Cordifolia is collected from local market from the Kolhapur. The raw materials and chemicals were taken from Ashokrao mane institute of pharmacy, ambap, kolhapur. All ingredients and excipients used are given in the Table.

**2. Method of preparation:**

Accurately weighed Carbopol 934 was taken in a beaker and dispersed in 50 ml of distilled water. Kept the beaker aside to swell the Carbopol for half an hour and then stirring should be done using mechanical/lab stirrer at 1200 rpm for 30 min. Take of propylene glycol and required quantity of Extract. Take propylene glycol in another beaker and add weighed quantity of propyl paraben and methyl paraben to it and stirred properly. After all Carbopol dispersed, 1 gm extract and preservatives solutions were added with constant stirring. Finally, volume made upto 100 ml by adding remaining distilled water and Triethanolamine was added drop wise to the formulations for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency.

**Formulation table:**

| Sr. No. | Ingredients | Batches | | | Role of Ingredient |
|---|---|---|---|---|---|
| | | A1 | A2 | A3 | |
| 1. | Ethanolic extract | 1 gm | 1g | 1g | Therapeutic agent |
| 2. | Carbapol 934 | 1.25g | 1.25g | 1.25g | Thickener |
| 3. | Propylene glycol | 12 ml | 12ml | 12ml | Humectants |
| 4. | Methyl paraben | 0.2gm | 0.2 gm | 0.2 gm | Preservative |
| 5. | Propyl paraben | 0.1gm | 0.1 gm | 0.1 gm | Preservative |
| 6. | Trienthanolamine | q.s. | q.s. | q.s. | Neutralizer |
| 7. | HPMC | 2gm | 2gm | 2gm | Active ingredient |
| 8. | Glycerine | 1 ml | 1 ml | 1 ml | Vehicle |
| 9. | Distilled water | Upto 100 ml | Upto 100 ml | Upto 100 ml | Vehicle |

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**Evaluation of gel:**

**1. Physical Evaluation :**

Physical parameters such as color and appearance were evaluated.

**2. Homogeneity:**

All developed gels were tested for homogeneity by visual inspection after the gel have been set in the container for their appearance and presence of any aggregates.

**3. pH** :

The pH of various gel formulations was determined by using digital pH meter. 2.5gm of gel was accurately weighed and dispersed in 25ml of distilled water and stored for two hours. The measurement of pH of each formulation was carried out in triplicate and the average values are represented. The pH of dispersions was measured using pH meter.

**4. Spreadability:**

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method Spreadability was measured on the basis of slip and drag characteristics of creams. An excess of gel (about 2 g) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. Weight of 1 kg was placed on the top of the slide for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 50 g. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 6.5 cm be noted. A shorter interval indicates better Spreadability.

Spreadability was calculated using the following formula:

**$S = M \times L / T$**

Where, S = Spreadability,

M = Weight in the pan (tied to the upper slide), L = Length moved by the glass slide and

T = Time (in sec.) taken to separate the slide completely each other.

**5. Viscosity :**

Viscosity of herbal gel was determined by using Brookfield rotational viscometer at 5, 10 20, 30 and 50 rpm using spindle no.64. Each reading was taken after equilibrium of the sample at the end of two minutes. The viscosity determination of samples was repeated three times

## III. RESULT AND DISCUSSION

**Collection and Authentication:**

The Roots were collected from the local farme from Kolhapur.

**Extraction of Rubia cordifolia:**

**Extractive values of Rubia cordifolia**

| Sample | Extraction Method | Solvent used | Wt. of sample | Extraction value (%w/w) |
|---|---|---|---|---|
| Rubia cordifolia roots powder | Soxhlet extraction | Ethanol | 50 gm | 20% w/w |

**Physicochemical evaluation of Gel:**

**1] Physical Appearance:**

**Physical appearance of gel**

| Sr. no. | Batch | Color | Appearance |
|---|---|---|---|
| 1 | A1 | Light Green | Green |
| 2 | A2 | Green | Green |
| 3 | A3 | Green | Green |

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**2] Homogeneity**

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container.

**Homogeneity of formulation**

| Sr. No. | Batch | Homogenecity |
|---|---|---|
| 1 | A1 | Homogeneous |
| 2 | A2 | Homogeneous |
| 3 | A3 | Homogeneous |

**4] Spreadability**

The time in seconds require to separate the two slides was taken as measure of spreadability.

**pH and Spreadability of leaves extracts formulation.**

| Sr. No. | Batch | pH | Spreadability (gm.sm/sec) |
|---|---|---|---|
| 1 | A1 | 6.85 /±0.03 | 15.19/±0.005 |
| 2 | A2 | 6.91 /± 0.03 | 14.80/±0.005 |
| 3 | A3 | 7.08 /± 0.03 | 15.73/±0.005 |

**5] Viscosity**

Viscosity of gel was determined by using Brookfield rotational viscometer at 5, 10, 20, rpm. Each reading was taken after equilibrium of the sample at the end of two minutes. The samples were repeated three times.

**Viscosity value of herbal gel**

| Sr. No. | rpm | Viscosity (Cps) |
|---|---|---|
| 1 | 5 | 3725 ±0.13 |
| 2 | 10 | 3914 ±0.27 |
| 3 | 20 | 4027 ±0.38 |

## IV. CONCLUSION

The findings of this study underscore the therapeutic potential of Leptadenia reticulata leaves, which exhibit substantial antimicrobial and antioxidant activities. The ethanolic extracts demonstrated comparable efficacy to established antimicrobial agents such as ciprofloxacin and amphotericin, while also showing significant antioxidant potential against standard compounds like Butylated Hydroxyl Anisole and L-ascorbic acid. These results suggest that Leptadenia reticulata could be a valuable source for developing herbal medicinal products with antimicrobial and antioxidant properties. Furthermore, the study highlights the importance of public education on the benefits of medicinal plants, aiming to foster greater acceptance and integration of herbal medicine into contemporary healthcare practices. This research supports the potential of Leptadenia reticulata in the Ayurvedic industry for formulating and marketing standard drugs with international acceptance, thereby contributing to the broader field of ethnomedicine and pharmacognosy.

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